

IN THE U.S. PATENT AND TRADEMARK OFFICE

APPLICANT(S): Kalle SAKSELA et al.
APPLICATION NO.: 09/579,894 GROUP: 1627
FILED: May 26, 2000 EXAMINER: B. Celsa
FOR: METHODS AND MATERIALS FOR GENERATING SH3
DOMAINS WITH TAILORED BINDING PROPERTIES

DECLARATION SUBMITTED UNDER 37 C.F.R. §1.132

Honorable Commissioner of Patents
Washington, D.C. 20231


I, Dr. Marius SUDOL do hereby declare the following.

I am an associate professor in Mt. Sinai School of Medicine, New York, NY. I am a researcher in the field of protein domains and I am an inventor on two USA patents. I obtained my Ph.D. degree at the Rockefeller University in New York City, USA. Dr. Kalle Saksela was a colleague of mine from the tenure at the Rockefeller University.

In the recent past I had a chance to analyze some of the patent proceedings of Dr. Saksela. Based on my analysis I would like to state the following: The capacity of the RRT-SH3 domains revealed in the publication from the Saksela lab (Hiipakka et al., 1999 J. Mol. Biol. 293:1097-1106), the subject matter of which corresponds to the above-identified patent application, is novel and cannot be directly compared to the finding published earlier by the laboratory (Lee et al., 1995, EMBO J. 14:5006-5015). In my judgement the idea of generating artificial, ligand-tailored SH3 domains became obvious only from the publica-

tion of Hiipakka et al., 1999 and not from the previous report of Lee et al. from 1995.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.


Dr. Marius SUDOL


Date

SENT BY MAIL TO:

MaryAnne Armstrong
BIRCH, STEWART, KOLASH & BIRCH, LLP
P.O. Box 747
Falls Church,
VIRGINIA, 22040-0747

An alignment showing approximately half of all SH3 domains found in the human genome. The positions of the five beta-strands of the SH3 protein fold are indicated with "b", and are separated by slashes ("/") corresponding to the loop regions or by a 3_{10} -helix ("3"). The first loop region is known as the RT-loop. The RT-loop consist of a region that is variable in sequence and length (typically six amino acids but ranging among the SH3 domains shown below from 3 to 14) flanked by four and eight relatively well-conserved residues. The variable region of the RT-loop (colored yellow) can be easily identified in the sequence of different SH3 domains based on the adjacent conserved amino acid motifs "ALYDY" and "DL" (colored green). The "ALYDY motif" almost invariably consists of a combination of the amino acids A/V - L - Y/F/W/H - D - Y/F/H, whereas the "DL motif", almost invariably consist of a combination of the amino acids D/E-L/I/V.

The present invention is based on our observation that by replacing the variable region of the Hck-SH3 domain of (EAIHHE; residues 69-74 of Hck protein) with completely random six-amino substitutions artificial SH3 domains (RRT-SH3 domains) can be generated that can bind with unnaturally high affinities to various ligands of natural SH3 domains despite the fact that their engineered RT-loop sequences bear no resemblance to natural SH3 domains. It is important to note, that we have successfully used Hck-derived RRT-SH3 domains to target with high affinity also ligands that (unlike HIV Nef) normally show no binding to Hck-SH3.

Although our Hck-derived RRT-SH3 library (carrying approximately 64 million different variants) appears sufficient for targeting almost any SH3 ligand protein (and thus to develop competitive inhibitors for virtually any SH3 domain), most if not all of the other SH3 domains could also be used as scaffolds for generating similar RRT-SH3 libraries. This could be done by replacing the variable part of the RT-loop (as defined above and colored yellow in the alignment below) by a stretch of random residues. Although this was the case in our Hck-derived RRT-SH3 library, the length of the randomized sequence would not need to be the same as in the original SH3 domain, and could range anywhere between 4 and 20.

ALYDY	DL	
V F F	E I	
W H	V	
H		
bbbbbb-----bbbbb-----bbbbb3333bbbbb		
IIVV ALYDY MEAE-----IH HD ELSFQKGDQMVVLEES-----GEWWKARSL-----ATRKEGYIPSNYVARV		Hck
TLFV ALYDY MEAE-----RT ED ELSFHKGEKFQILNSS-----EGDWWEARSL-----TTGETGYIPSNYVAPV		Fyn
ELVL ALYDY QOE-----KSPREVTMKKGDIITLLNST-NKARHREWLCDWWKVEVN-----DRQGFVPAAYVKKL		alpha-II spectrin
QRVM ALYDY FOA-----RSPREVTMKKGDIITLLSSI-----NKDWWKVEAA-----DHQGI VPAVYVRRRL		alpha-I spectrin
NLVI ALYDY MEP-----SHD GL GFEKGEQLRILEQS-----GEWWKAQSL-----TTGQEGFIPFNFVAKA		Lck
DIVV ALYDY PDG-----IHP DL SPFKKGEKMKVLEEH-----GEWWKAKSL-----LTKKEGFIPSNYVAKL		Lyn
HVVO ALYDY PSS-----SNDE EL INFEKGDVMDVIEKP-----ENDPEWWKCKRI-----NGMVGVLVPKNYVTVM		Nck1 #3
PQCK ALYDY EEV-----KDKEADKDC LP FAKDDVLTIVIRRV-----DENWAEGLMA-----DKIGIFPISYVEFN		POSH#2
EWCE ALYDY SETA-----ETSD EL SPFKRGDRIQILRL-----DSDWCRGR LQ -----DREGIFPAVFVRPC		SH3d19 #4
MEAV ALYDY ETA-----SGE DL LSFHTGDVLKILSNQ-----EEWFKAE LG -----SQEGYVVPKNFIDIQ		GADS/Grp2 #1
PHAV ALYDY DEPA-----EQVD DL NLTSGEIVYLLEKI-----DTDWYRGNCR-----NQIGIFPANYVKVI		SH3d19 #2
RIFV ALYDY DPVSMSPNPDAGEE EL PFREGQILKVP GD K-----DADGFYQGE GG -----GRTGYIPCNMVAEV		PRAX-1 #2
NTYV ALYDY KFVP-----QENE DL EMRPGDIITLLEDS-----NEDWWKKG KI Q-----DRIGFFPANFVQRL		Stac
TI FV ALYDY MEAE-----RTTE DL SPFKKGERFQIINNT-----EGDWWEAR SI -----ATGKNGYIPSNYVAPA		Yes
SQVE ALYDY SYEA-----TQPE DL EFQEGDIILVLSKV-----NEEWLEGE CK -----GKVGIFPKVFVEDC		p67-phox #2
IEVT ALYDY SEEG-----QQPG DL INQAGDRITVISK T -----DSHFDDWEGK LR -----GQTGIFPANYVTMN		Homology to Grb2
HFVV ALYDY MTA-----MND DL QMLKGEKLQVLKGT-----GDWWLAR SL -----VTGREGYVPSNFVARV		Blk
TTFV ALYDY YES-----RTTE DL SPFKKGERLQIVNNT-----RKVDVREGDW LA HSL-----STGQTGYIPSNYVAPS		N-Src
TTFV ALYDY YES-----RTTE DL SPFKKGERLQIVNNT-----EGDW LA HSL-----STGQTGYIPSNYVAPS		Src
HSMV ALYDY ANP-QESSPNMDVEA EL PFRA GD VITVFGGM-----DDDG FY YGE LN -----GQRLVPSNFLE GP		PRAX-1 #3
VCAE ALYDY BHVT-----MDD DL Q LG FKAGDVIEVMDAT-----NREWWWR VA -----DGEGWFPASFVRLR		Asef / ARHGEF4
IQV ALYDY DEL P -----REPC NL ALRAAEY LI LEKY-----NPHWWKAR DR -----LGNEGLIPSNYVTEN		Txk
VCAE ALYDY BHVT-----MDD DL Q LG FKAGDVIEVLEAS-----NKDWWWR SE -----DKEAWFPASFVRLR		Homology to Asef
VSAE ALYDY BHVT-----MAN REL AFKAGDV IK VL DA S-----NKDWWWQ ID -----DEEGWFPASFVRLW		ARHGEF9
GVIY ALYDY MEP-----QND DL ELPMKEGDCMTI HR E-----DEDEIEWWAR LN -----DKEGYVPRNLLGLY		53BP2
GVAY ALYDY MEAE-----QNS DL ELSPHEGDA LT ILRRK-----DESETEWWAR LG -----DREGYVVPKNLLGLY		ASPP1
GAVY ALYDY KSA-----EFG DL ELSFREGESVTVLRRD-----GPEETDWWWA AL H-----GQEGYVPRNYFG LF		RAI
PTVV ALYDY MTA-----NRSE DL TIHRGD IR VFFK D -----NEDWWYGS IG -----KGQEGYFPANHVASE		Homology to Tipd
RKAR ALYDY MDA-----ANST EL SLLADEVITVFSV V -----GMSD SL WLMGERG-----NQKGKVPITYLE LL		endophilin B1
RKAR ALYDY MEAE-----ADS SL ELALLADELITVYSL P -----GMDPDW LI GERG-----NKKGKVPITYLE LL		endophilin B2
RKAK ALYDY BERG-----ENE DL ELSPKAGDIIT EL ESV-----DDDWM S GELM-----KSGIFPKNYIQ FL		SH3d19 #5
EEYI ALYDY FPSS-----VEP DL ELTFTEGEEILV TQ KD-----GEWW TG SIG-----DRSGIFPSNYVK PK		Intersectin 2 #3
GIAI ALYDY DECA-----RDM REL SL LG DVVVKIY TK M-----SANGWWR GE VN-----GRVGWFPSTYVE ED		Vav3 #2

NMFVALLHSYSA	-----HGPDELDLQKGEGRVVLGKC	-----QDGWLRGVSL	-----VTGRVGIFPNNYVIPI	Predicted protein
GTAKARMDECA	-----RDRSLSLKEGDI I KILNKK	-----GQQGWWRGEIY	-----GRVGVFPANYVEED	Vav1 #2
LLARALMNCNP	-----DCSDELAFSRGDILTILEQH	-----VPSEGWKCLLH	-----GRQGLAPANRLQIL	HEFL
VRVRALMDYDG	-----QEQDELFSKAGDELTKLGEE	-----DEQGWCRGRLD	-----SQQLGLYPANYVEAI	PACSIN1
VRVRALMDYEG	-----QEHDLSFKAGDELTKMEDE	-----DEQGWCKGRLD	-----NGQVGLYPANYVEAI	PACSIN2
IPIKAIICDMRQ	-----IETIYKDDCEVLANS	-----HRAKWKVISPT	-----GNEAMVPSVCFVTP	BPAG1
PRCRALMDYVG	-----QDVDELSFNVEVIEIIMED	-----PSGWWKGRLLH	-----GOEGLFPNGYVEKI	MYOSIN I-f
YTAVALMDYQA	-----AGDDELSFDPDDIITNIEMI	-----DDGWWRGVCK	-----GRYGLFPANYVELR	Cortactin/EMS1
ISAVALMDYQG	-----EGSDELSFDPDDIVTDIEMV	-----DEGWWRGRCH	-----GHFGLFPANYVKLL	LckBP1 / HS1
NVYLALMAMKP	-----QKSDLELHKGEYRVLEKC	-----QDGWFKGASL	-----RTGVSGVFPNGYVTPV	FLJ00204 #1
TKARVMDFAA	-----EPGNNELTVNAGEI IITINPD	-----VGGGWLEGRNI	-----KGERGLVPTDYVEIL	Sorting nexin 9
LRARALMDERS	-----ENPGMISLREHEVLSLCSAQ	-----DIEGWLEGVNS	-----RGDRGLFPASYVQVI	Sorting nexin 18
RRMVALMDYDP	-----RESSPNVDVEAEITFTCTGDI I TVFGEI	-----DEDFYYGELN	-----GQKGLVPSNFLEEV	KIAA0318 #3
RRAKALLDFER	-----HDDDELGFPRKNDI I TVSQK	-----DEHCWVGELN	-----GLRGWFPKAFVEVL	Homology to EEN-B1
VEAIMEEDYQA	-----QHDDELITISVGEI I TNIRKE	-----DDGWWEQGIN	-----GRRGLFPDNFVREI	CIN85 #1
VDYIMEEDYDA	-----VHDDELITIRVGEI I RNVKKL	-----QEGWLEGEIN	-----GRRGMFPDNFVKEI	CMS #1
HYFVALMDYQA	-----RTAEDLSFRAGDKLQVLDL	-----HEGWWFARHL	-----EKRRDGSSQQLQGYI PSNYVAED	Frk/Rak
VLAKALMDNVA	-----ESPDELSFRKGDIMTVLEQD	-----TQGLDGWVLCSLH	-----GRQGI VPGNRLKIL	p130Cas
LMARALMDNVP	-----ECAEELAFRKGDI I TVIEQN	-----TGGLEGWVLCSLH	-----GRQGI VPGNRVKLL	HEFL/CasL
QLARALMDNTA	-----ESPOELSFRRGDVLRVLQRE	-----GAGGLDGWVLCCLSH	-----GQGI VVPANRVKLL	Efs/Sin
FSYQALMDYIP	-----QNDDLELRLDGDIVDMVEKC	-----DDGWVFGTSR	-----RTKQFGTFPGNYVKEL	Ponsin #3
EPFOALMDMTP	-----RNEDELELRESVDIVMEKC	-----DDGWVFGTSR	-----RTKFFGTGTFPGNYVKEL	ArgBP2a #3
RTYRAMMDYSA	-----QDEDEVSFRDGDIVNVQPI	-----DDGWMYGTVQ	-----RTGRTGMLPANYIEFV	Nebulette
KRYRAMMDYSA	-----ADEDEVSFDQGDIVNVQOI	-----DDGWMYGTVE	-----RTGDTGMLPANYVEAI	Lasp-1
KI FRAMMDYMA	-----ADADEVSFKDGDIVNVQAI	-----DEGWMYGTVQ	-----RTGRTGMLPANYVEAI	Nebulin
VVARALMDFAA	-----VSEETISFRAGDMLNLALKE	-----QQPKVRGWLLASLD	-----GQTTGLIPANYVKIL	Peroxin-13
VYYRALMDPES	-----RSHDEITIQPGDIVMVKGW	-----VDESQTGEPGLWGBELK	-----GKTGWFPANYAEKI	Intersectin 1L #1
VYYRALMDPES	-----RSHDEITIQPGDIVMVDESQ	-----TGEPGLWGBELK	-----GKTGWFPANYAEKI	Intersectin 1 #1
VNYRALMDPEA	-----RNHDEMSFNSGDI IQVDEKT	-----VGEPGLWLYGSFQ	-----GNFGWFPNPNYVEKM	Intersectin 2 #1
PVWTALMDYEP	-----SGQDELALRKGDREVLRSR	-----AAISGDEGWAGQVQ	-----GQVGI FPNPNYVSRG	Mlk3
RLCKALMSFOA	-----RQDDELELKEGDIVI I HEKK	-----EEGWVFGSLN	-----GKKGHFPAAYVEEL	Nostrin
FKVQALMDYTA	-----TDTDELELQKAGDVVLVIFPQ	-----NPPEQDEGLWGVKESDWNQHKELEKCRGVFPENFTERV	-----	Amphiphysin II
YKVEITHDEFA	-----ANSDELELQRGDVVLVVPDS	-----SEADQDAGVLWGVKESDWNQHKELEKCRGVFPENFTERV	-----	Amphiphysin
RQCKALMDYIP	-----QNEDELELKVGDIDINEEV	-----EEGWWSGLTN	-----NKLGLFPNPNYVKEL	CMS #2
QEYRALMDYTA	-----QNPDELDLSAGDILEVILEG	-----EDGWWTVERN	-----GQRGVFPNPNYVKEL	CD2BP1
PVWTALMDYEA	-----AGDELELTLRRGDRVQVLSQD	-----CAVSGDEGWWTGQLP	-----SGRVGVFPNPNYVAPG	Mlk2
VRVRALMDYAG	-----QEADELSFRAGEELLKMSSE	-----DEQGWVCGQLQ	-----SGRIGLYPANYVECV	PACSIN3
YQYRALMDYKK	-----EREEDDLHLGDILTIVNKGSSDQGEARPEEIGWLNNGYNE	-----TTGERGDFPGTYVEYI	-----	P85A
FQYRALMDYER	-----ERPDELELPGDVVLSRAAEGGERCPQSVGWMPGLNE	-----RTQRGDFPGTYVEFL	-----	P85B
LKGRALMDYHS	-----ENKEETSIQDEDLVIFSET	-----SLDGWLVGQNS	-----RGETGLFPASVVEIV	Predicted protein
PQCKALMDYDA	-----QDTDELSFNANDI I DI IKED	-----PSGWTGRLR	-----GKPGLPNPNYVTKI	MYOSIN I-C
KKVVALMDYMP	-----MNANDELQLRKGDEYFILEES	-----NLPWWRARDK	-----NGQEGYIPSNYVTEA	Btk
TVVIALMDYQT	-----NDPOELALRRNEEYCLLDSS	-----EIHWWRVQDR	-----NGHEGYVPSYLVKE	Itk
KYVKILMDYTA	-----RNANELSVLKDEVLVLELDG	-----RQWVKLRSR	-----SQAGYVPCNILEGA	Eps8R2
RIFVALMDYDPLTMSPNPDAAEELPFKEGQI I KVYGDK	-----DADGFYRGETC	-----ARLGLIPCNMVSEI	-----	KIAA0318 #2
VVVVALMDYVA	-----QQEQELDIKKNERLWLLDSS	-----KSNWRVRNS	-----MNKTGFVPSNYVERK	Nck1 #1
VIVIAKMDYTA	-----QQDQELDIKKNERLWLLDSS	-----KTWWRVRNA	-----ANRTGYVPSNYVERK	Nck2 #1
MESVALMSFOA	-----TESDELA FNKGDITLKI LNME	-----DDQNWYKAELE	-----GVEGFIPKNYIRVK	Grap #1
LKVRALMDYFN	-----LHDPTALNVRAGDVIITVLEQH	-----PDGRWKGH I H	-----ESQRGTDRIGYFPNGIVEV	CASKIN #2
RKVRALMDYEA	-----AEDNELTFKAGEI I TVLDSS	-----DPNWWKGETH	-----QGIGLFPSNFVTAD	STAM1
RKVRALMDYEA	-----VEDNELTFKHGEI I TVLDSS	-----DANWVKGENH	-----RGIGLFPSDFVTN	STAM2
TYVQALMDYEDP	-----QEDGELGFRRGDFIHMNDNS	-----DPNWWKGACH	-----GQTGMFPRNYVTPV	Grb2 #2
TYGVALMDYFOA	-----LEPNELDFEVGDKIRILATL	-----EDGWLEGLSK	-----GRTGIFPRYFVKLC	KIAA1010 #4
PCCRALMDYEP	-----ENEGELGFKEGDI I TLTNQI	-----DENWYEGMLH	-----GHSGFPPINYEVL	EEN-B1/SH3GL2
PCCRGLMDYEP	-----ENQGELEGFKEGDI I TLTNQI	-----DENWYEGMIH	-----GESGFPPINYEVI	EEN-B2/SH3GL3
PSCKALMDYEP	-----ENDGELGFHEGDVIITLTNQI	-----DENWYEGMLD	-----QSGGFPLSYVEVL	EEN/SH3GL1
RPARAKMDYKA	-----QTLKELPLQKGDIVYIYKQI	-----DQNWYEGEHH	-----GRVGIFPRTYIELL	Ponsin #1
KAARLKMDYFOA	-----QSPKELTLQKGDIVYIYKEV	-----DKNWLEGEHH	-----GRLGIFPANYVEVL	Vinexin #1
LPAKALMDYKA	-----QTSKELSFKKGDTVYIILRKI	-----DQNWYEGEHH	-----GRVGIFPISYVEKL	ArgBP2a #1
PKYVGLMDYKS	-----RTDELSFRAGDVVFHVARKE	-----EQWWATLLD	-----EAGGAVAQGYVPHNYLAER	Brk
LKMOVLMDEFA	-----RNPRELTVVQGEKLEVLDS	-----KRWWLVKNE	-----AGRSYIPSNILEPL	Eps8R3
PCAKALMDYEG	-----KEPGELKFSKGDIIILRRQV	-----DENWYHGEVN	-----GIHGFFPTNFVQII	POSH #1
SVVRAIMDEFCP	-----SVSEELPLFVGDI I EVLAVV	-----DEFWLLGKKE	-----DVTGQFPSSFVEIV	KIAA1010 #1
RWARALMDYEA	-----LEDDELGFHSGEVVEVLDS	-----NPSWWTGRLH	-----NKLGLFPANYVAPM	GADS/Grp2 #2
EYVRALMDYFNG	-----NDEBELPFKKGDILIRIDKP	-----EEQWNAEDS	-----EGKRGMI PVPYVEKY	Crk #1
EYVRTLMDEFG	-----NDABELPFKKGEI I LVIEKP	-----EEQWWSARNK	-----DGRVGMIPVPYVEKL	CrkL #1
IEAIAKMDYVG	-----RTARELSFKKGASLLLYQRA	-----SDDWWEGRHN	-----GIDGLIPHQYIVVQ	SRGAP2
IEAIAKMDYVG	-----RSARELSFKKGASLLLYHRA	-----SEDWWEGRHN	-----GIDGLVPHQYIVVQ	SRGAP1
IEAIAKMDYMG	-----RSPRELSPFKKGASLLLYHRA	-----SEDWWEGRHN	-----GVDGLIPHQYIVVQ	SRGAP3
CQVIAMMDYAA	-----NNEDELFSKQGLINVMNKD	-----DPDWWQGEIN	-----GVTGLFPPSNYVKMT	Intersectin 2 #5
CQVIAMMDYAA	-----NNEDELFSKQGLINVMNKD	-----DPDWWQGEIN	-----GVTGLFPPSNYVKMT	Intersectin 2 #5
RTVKALMDYKA	-----KRSDELSFCRGALIHNVSK	-----PGGWKGDYD	-----TRIQQYFPPSNYVEDI	PLC-gamma-2
CAVKALMDYKA	-----QREDELTFIKSAI I QNVEKQ	-----EGGWWRGVDYD	-----GKKQLWFPNPNYVEEM	PLC-gamma-1
LCARALMDYQA	-----ADDTLSFDPENLITGIEVI	-----DEGWWRGYGP	-----DGHFGMFPANYVELI	HIP-55
EKVVALMDYTK	-----DKEDLSFQEGAI I YVICKN	-----DDGWYEGVMN	-----GVTGLFPNGYVESI	Ab1BP3
EKVVALMDYTK	-----DKDDELSFMEGAI I YVICKN	-----DDGWYEGVCN	-----RVTGLFPNGYVESI	Abi-1
GLWALMDYEA	-----RGEDELTLRRGQLEVEVLSQD	-----AAVSGDEGWAGQVQ	-----RRLGIFPANYVAPC	Mlk4

EAHRVLEGGVP-----ETKEELQVMPGNIVFVLKKG-----NDNWATVMFN-----GQKGLVPCNYLEPV

p67-phox #1